# WOUND HEALING PROCESSES IN AMPUTATED MOUSE DIGITS[1]

OSCAR E. SCHOTTÉ AND CHARLES B. SMITH

*Department of Biology, Amherst College, Amherst, Massachusetts*

For obvious reasons the healing of wounds has been the object of study by many biologists and medical researchers. The literature is enormous and basic information concerning the subject can be found in many excellent reviews by Marchand (1901), Arey (1936), and Cameron (1952), and has been incorporated in text books of Histology, Maximow and Bloom (1957), Surgery, Harkins (1957) and Pathology, Robbins (1957).

Difficulties inherent in mammalian material and the desire for uniform techniques in the study of wound healing have caused the non-clinical investigations to be concentrated upon a limited variety of wounds. Studies of epidermal and dermal healing have been generally confined to skin wounds made on the backs of rats, Lindquist (1946) or on rabbit ears, Clark and Clark (1953), and Levander (1950). The important problem of bone repair and regeneration has been studied predominantly in rib or leg fractures in rabbits, Ham and Harris (1956), cats, Blaisdell and Cowan (1926) or rats, McLean and Urist (1955).

But when one considers the problem of the type of wound that occurs after amputation of an appendage it is surprising to discover a lack of fundamental information of the subject. Systematic post-amputational wound healing has heretofore been studied only in the lower vertebrates, probably because of their capacity to regenerate limbs. Studies in man have been confined to gross observations and to attempts to hasten the process of healing via skin grafts and other devices (Slocumb and Pratt, 1944). Occasional studies have appeared in the literature that dealt with amputational healing in rat (Nicholas, 1926) or sheep fetuses (Barron, 1945); however, even these biologists confined themselves to gross observations. We have been unable to find any description concerned with the histological process of post-amputational wound healing in mammals.

This paper constitutes an attempt at a systematic investigation of the histological aspects of wound healing processes occurring in amputated mouse digits. Our objectives are two-fold: first, to describe the post-amputational events that lead to simultaneous and coordinated repair of skin, connective tissue and bone; secondly, to evaluate the findings from the point of view of the student of regeneration, and to ascertain whether there are any aspects of this process that might be comparable to those seen in regenerating vertebrates.

## Materials and Methods

The animals used in this study were four- to eight-week-old Swiss mice of a strain purchased from the Roscoe B. Jackson Laboratory in 1953 and maintained at this laboratory to the present time. They were fed a diet of Purina Lab Chow

[1] Supported by Grant C-2236 from the National Institutes of Health.

and kept at a temperature of 80° (± 2° F.) in plastic cages in groups which never exceeded seven mice per cage.

Previous attempts at a systematic histological study of post-amputational wound healing in mammals conducted at this laboratory (McIntosh, Amherst College Honor's Thesis, 1954) were confined to amputations made through the carpal, metacarpal or radius-ulna regions of the forelimb. It was found, particularly in the younger individuals, that the many bones at the amputation site were often severed at different stages of ossification, surely a complicating factor in an histological study. It is also reasonable to assume that a long bone amputated through the diaphyseal region might present a healing pattern different from the one amputated through the epiphysis. It was therefore decided to select for study the digit containing within each particular segment a single bone at a definite stage of development. The digits of the forelimb, because of their small size (roughly one millimeter in diameter), are ideally suited for an experimental and histological study.

The digits to be amputated were identified with the numbers I to V starting with the short digit which is homologous to the human thumb. The phalanges are numbered 1 to 3 from proximal to distal. Phalanx 3 is quite small and almost completely covered with a nail, so digital segments containing the longer phalanx 2 of the second, third and fourth digits of both forelimbs were selected for levels of amputation. Using the above numeration, a code system was devised to identify the amputation stump and host animal. Thus, for example, the third digit from the left forelimb of Case MCS 50, amputated through the second phalanx, was designated in our records and identified in this paper as Case MCS 50, L-2-III.

The mice were narcotized for the initial amputation with ether. Before fixation of the amputation stump, the animals were narcotized with a subcutaneous injection of veterinary Nembutal (Pentobarbital Sodium, Abbot) permitting a deeper narcosis. The doses varied from 1.8 mg. to 2.4 mg. depending upon the age, size and condition of the animal. The amputations were performed with small surgical scissors under the dissecting microscope. Due to considerable bleeding, no attempt was made to trim the bone stump to the level of the soft tissues; it is probably for this reason that in some cases a protruding bone complicated the healing processes. To avoid using an excessive number of animals, four to six digits were amputated on the same individual and these amputations were done either simultaneously or at varying intervals.

Before fixation, the digital hair was removed with a commercial depilatory (Nair). The stump of the digit was severed at the metacarpophalangeal joint and fixed in Bouin's solution for three days or longer and decalcified in Jenkin's solution for periods ranging from six to fourteen days. The embedding procedures entailed three paraffin changes and a 24-hour perfusion in the last paraffin bath at 56–58° C. All digits were sectioned at 10 micra. Difficulties in sectioning encountered were attributable to incomplete decalcification. Most sections were stained with Harris's modification of Delafields' hematoxylin and counterstained with orange G. Mallory's polychromatic stain was also used to show development of connective tissue fibers.

This investigation is based upon histological studies from 191 digits fixed at various times ranging from six hours to seven weeks after amputation.

## Experimental Results

Although, as mentioned in the introduction, the role of the epidermal, dermal and bony tissues in the healing of wounds has been well studied, it was desirable to re-investigate all these processes in the healing of amputational wounds. The healing stumps were fixed and studied histologically at six-hour intervals for the

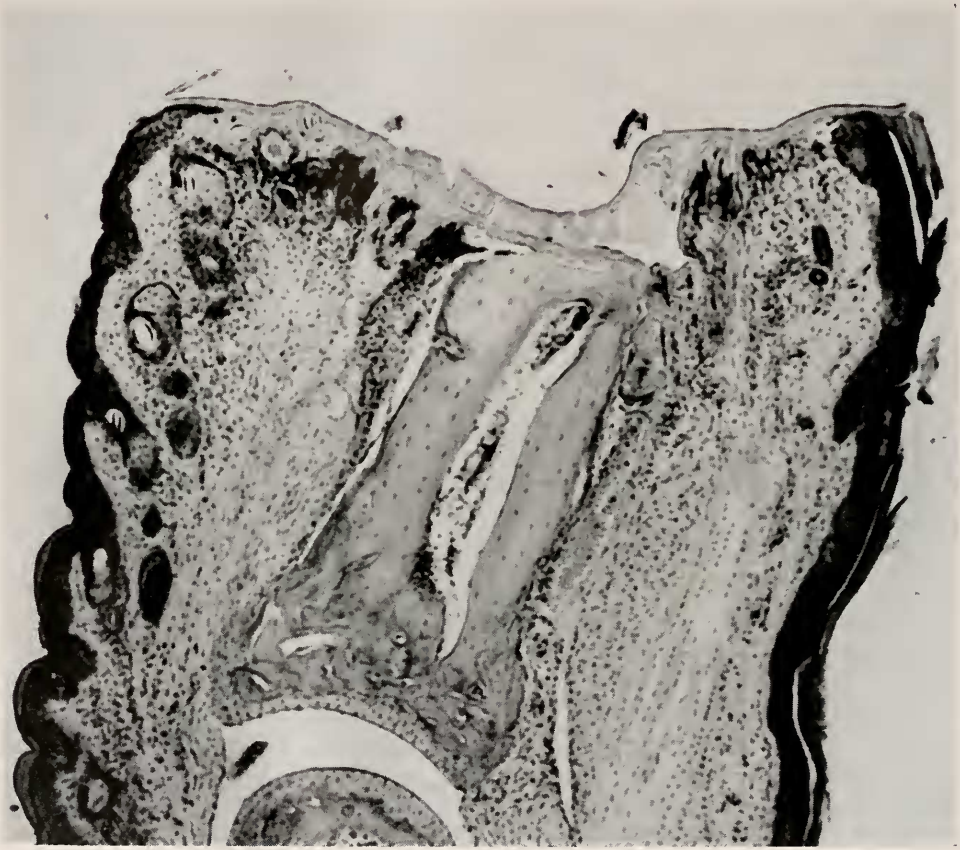

Figure 1. Photomicrograph of a sagittal section from a digit (Case MCS 52, L-2-III) fixed six hours after amputation. This case illustrates several aspects characteristic of the early wound healing process: (a) the entire surface of the cut digit is covered by a layer of blood coagulum with only a few blood cells in it; (b) beneath this coagulum can be seen large masses of white blood elements characteristic of this early inflammation phase; (c) the cut edges of skin show the beginning of epithelial migration and healing (90×).

first two days, and daily thereafter for three weeks. The healing of epidermis, dermis and bone as observed in these amputational wounds will be discussed with particular emphasis upon the unique interrelationships between these tissues.

For aid in orientation when viewing the figures, a sagittal section of a mouse digit illustrating its typical anatomy is presented in Figure 1 (Case MCS 52, L-2-III). The skin on the right side of the photomicrograph represents the volar

surface, the left side representing the dorsal surface. The palmar or volar surface of the mouse digit is characterized by the absence of hair follicles and by a thick epithelium and dermis, while the dorsal epithelium possesses many hair follicles and is thinner than the volar. When a digit is sectioned horizontally the follicle distribution permits one to determine the exact position of the section. Amputation in this case was made through the mid-diaphyseal region of phalanx 2, and it is possible to observe the retracted extensor and flexor tendons on both sides of the bone.

*The early hours of wound healing after amputation* are characterized, according to Arey (*op. cit.*) and Robbins (*op. cit.*) by provisional closure with a blood clot and subsequent inflammation. Six hours after amputation (Fig. 1) the wound


FIGURE 2. Photomicrograph of longitudinal section from a digit (Case MCS 129, L-2-III), fixed one day after amputation. A continuous layer of epithelial cells covers the amputation surface and it separates from the stump the large scab composed of clotted blood and various tissue debris. Inflammation at this stage has spread into the stump, and large masses of leukocytes have accumulated around the cut end of the bone (150×).

area is covered by a solidified blood clot beneath which polymorphonuclear leukocytes are agglomerating. By one day (Fig. 2, Case MCS 129, L-2-III), the extent of necrosis as indicated by the area of leukocytic activity has spread deeper into the tissues, particularly around the cut end of the bone. The polymorphonuclear leukocytes that characterize the early stages of inflammation begin to die off and they become extruded with part of the scab (Fig. 2). Subsequently monocytes and lymphocytes from the blood and young macrophages from the tissues identifiable in large numbers for at least three days replace the polycytes.

*The first stages of epithelial migration* and healing are in most aspects similar to those first described by Loeb (1898) in his classic work on epithelial wound healing. As seen in a digit fixed six hours after amputation (Fig. 3, same case as Fig. 1), the first cells to migrate derive generally from the granular layer

of the epithelium. These cells rapidly swell, elongate and begin to migrate into and over the newly formed clot. The "syncytial protoplasmic layer" which according to Loeb (*op. cit.*) covers the clot or scab cannot be confirmed from the evidence of Figure 3, and from all the other sections we have studied. Rather, we have repeatedly observed an exudate (clearly shown in Fig. 3) of the type to which Weiss in several of his papers attracts attention (see last report on the subject, Weiss, 1959). We have made no histochemical study of the constitution of this

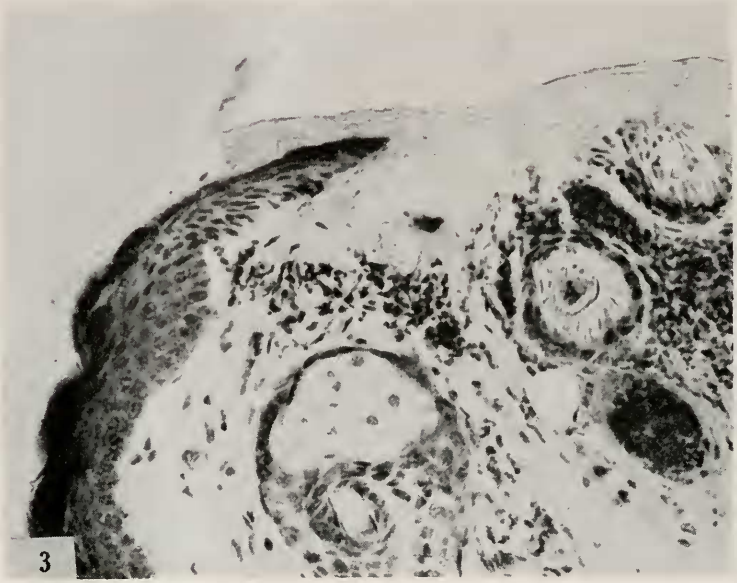


FIGURE 3. Photomicrograph of the upper left amputation site of Case MCS 52, L-2-III (shown in Fig. 1). The early migration six hours after amputation of the epithelium from the dorsal skin edge is shown. The migratory elements derive from the granular and lower horny layers of the epithelium, and the migrating wedge of cells can be seen invading the acellular exudate exhibiting fibrous structure. Beneath the clot large numbers of inflammatory leukocytes surround the hair follicles (300×).

exudate, but the photomicrograph clearly shows its orientational patterns (parallel to the amputation surface) so important for "contact guidance" (Weiss, *op. cit.*) of epithelial cells migrating over a semisolid substrate.

The migration from the basal Malpighian layer proper takes place less rapidly. At six hours these cells are just beginning to swell and to elongate, and it is not until eighteen hours after amputation that a migrating wedge of epithelium from the Malpighian layer can be observed to infiltrate the area beneath the scab and the tissue debris. Once started this migration continues more rapidly, since one day after amputation a continuous layer of epithelial cells from the Malpighian layer can be found covering the area of tissue debris underneath the scab (Fig. 2). It is, however, only exceptionally that the epithelial covering is completed within one day. As a rule epithelial healing is observed in the majority of the digits only three days after amputation (eight out of the thirteen cases studied showed

at three days a complete epithelial covering of the stump), in some other digits, undoubtedly due to protruding bone debris, this process is still further delayed. Complete epithelial repair is shown in Figure 4 (Case MCS 55, R-2-II) where the new epidermis shows early stratification. Also shown on the figure is a continued proliferation and migration of the Malpighian layer resulting in this and in other cases in a thick (12 cell layers) epithelial wedge over the cut end of the bone (see also Fig. 5, Case MCS, R-2-III). This local or general thickening of

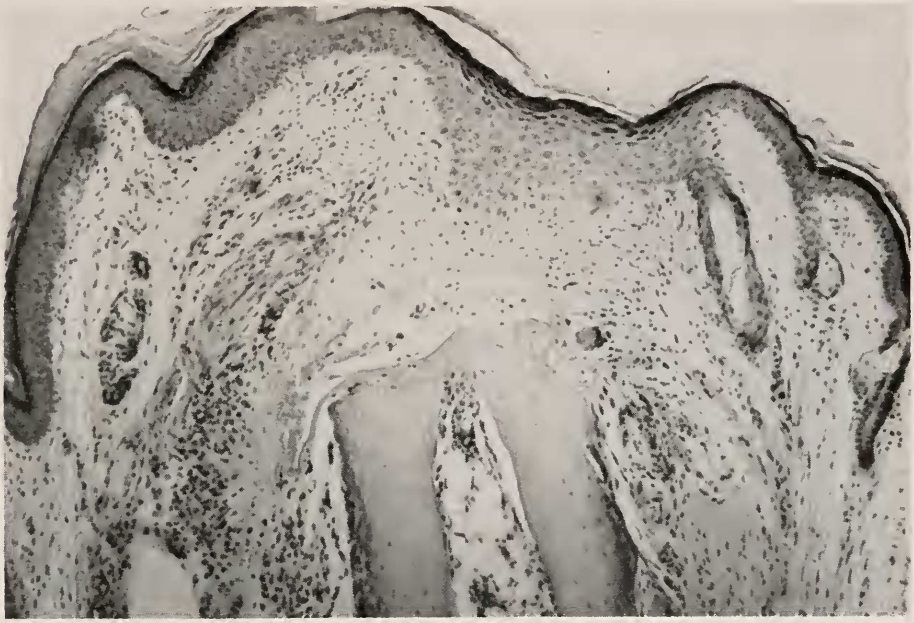

FIGURE 4. Photomicrograph of a longitudinal section from a digit (Case MCS 55, R-2-III), fixed three days after amputation and showing complete epidermal healing. Note: (a) the differentiation of epidermis into several distinct layers and the epidermal wedge with loosened cellular elements extending toward the bone; (b) between the covering epithelium and the bone are amorphous masses of clotted blood and tissue debris, while to the left of the bone masses of necrotic tissue can be seen; (c) individual fibroblasts identifiable by their spindle shaped form can be seen migrating anteriorly on both sides of the bone shaft; (d) the circular orientation of the tissues and the small area of epithelial proliferation, ahead of the phalanx reflects the contraction of the amputational wound (110×).

epidermal layers after amputation is a feature which closely resembles that described by Rose (1948) as a regular feature of epidermal growth in early regeneration in the newt.

*The reconstruction of dermal tissues* begins three days after amputation. Fibroblasts from surrounding connective tissue and particularly from the regions of cut tendons can be seen streaming into the wound area. They proceed to intermingle with the pool of red cells and tissue debris that is characteristically found between the cut end of the bone and the new pad of epithelium. Once started, the proliferation and migration of fibroblasts proceeds rapidly and by six days (Fig. 5) a completed cap of fibroblasts separates the epithelium from the cut end

of the bone. Seven days after amputation 13 of the 15 cases studied showed a well defined dermal pad.

Our observations of dermal healing concur with the previous descriptions of Arey (*op. cit.*) and Robbins (*op. cit.*): new blood vessels with at first indistinct walls accompanying the migrating fibroblasts become evident. Also, the first new collagenous fibers are seen at seven days in preparations with Mallory stain; consequently, the sub-dermal tissues become increasingly dense and fibrous until about two weeks after amputation when a characteristic dermal pad is found surrounding the end of the bone and the newly formed callus.

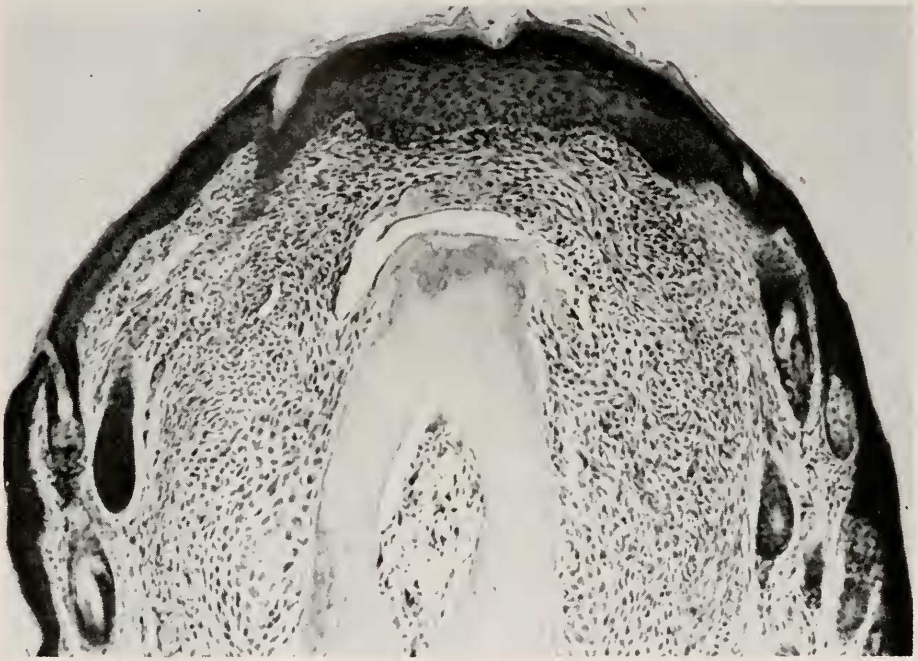

FIGURE 5. The photomicrograph of a longitudinal section from a digit (Case MCS 1, R-2-III) fixed six days after amputation shows: (a) the thick epithelial covering; (b) the connective tissue fibroblastic invasion into the amputation area resulting in a sizeable cap consisting of numerous layers of connective tissues; (c) the bursa-like cavity between this dermis and the end of the bone; (d) the extensive proliferation of periosteal elements with a large mass of cartilage cells on both sides of the bone (125×).

*Reconstruction of bone tissue* is also seen for the first time at three days, and our observations of the early stages of repair agree with the descriptions of McLean and Urist (*op. cit.*). The trauma of the operation and particularly the disturbance of vascular supply results in the death of osteocytes for varying distances from the distal end of the cut bone toward its proximal region. Empty bone lacunae such as shown in Figures 4 and 5 are represented under higher magnification in Figure 6 (Case MCS 100, R-2-III), fixed three days after amputation. The empty lacunae occupy most of the bone shaft; however, near the periosteum surviving osteocytes are discernible. The existence of surviving osteocytes at the proximal end of the

bone shaft indicates that necrosis affects only the osteocytes located within the distal area of the bone affected by amputation. We have observed that the dead bone matrix is subsequently destroyed and removed by macrophages and giant cells, while the remaining bone tissue undergoes osteoclastic reorganization, which is particularly active around six days after amputation.

Reconstruction of new bone and the formation of a callus begins about three days after amputation as a proliferation of the osteoblasts forming the endosteal and periosteal coverings of the bone (Fig. 6). Once started, the proliferation of these osteogenic cells proceeds quite rapidly and by six days (Fig. 5) the early stages of callus differentiation become noticeable. On other sections not here


FIGURE 6. Photomicrograph of phalanx 2 from a digit (Case MCS 100, R-2-III) fixed three days after amputation showing: (a) the dead tip of the bone recognizable by the empty bone lacunae, while more proximally and toward the periosteal edges live osteocytes are visible; (b) the proliferation of the osteogenic cells in the periosteum on both sides of the bone (240×).

illustrated, new bone trabeculae may be seen next to the old bone tissue, while the elements farthest away from the bone retain a fibroblast-like appearance. The cells which remain in an intermediate position generally go through a cartilage cell stage which is well illustrated in Figure 5 (to the left side of the shaft).

The size and shape of the callus that is formed varies according to the level of amputation. Observations indicate that amputation through the diaphysis of the bone results in a large callus with a characteristic intermediate cartilagenous stage, while if the epiphyses are transected a much smaller callus results. At the former level new bone trabeculae originate first near the bone and then at the outer edge of the callus. The cartilage cells intermediate between these two levels either disintegrate to make room to a highly vascularized zone that closely resembles the former hematopoietic marrow cavity, or they become calcified to leave thin bone trabeculae connecting the new outer shell of the stump to the old bone.

The formation of a new callus collar around the diaphyseal shaft is best shown in a cross-section of a thirteen-day-old amputation stage (Fig. 7, Case MCS 1, L-2-III). The outermost layers of osteogenic cells appear to be the largest and most active. Between the outer layer of new bone trabeculae and the old bone shaft can be seen perpendicularly arranged trabeculae, cartilage cells, and blood elements. Not indicated in this figure, but nevertheless observable in many sections, is a final callus that is much larger and of a more amorphous appearance than that typically described for healing in fractures (Ham and Harris, *op. cit.*).

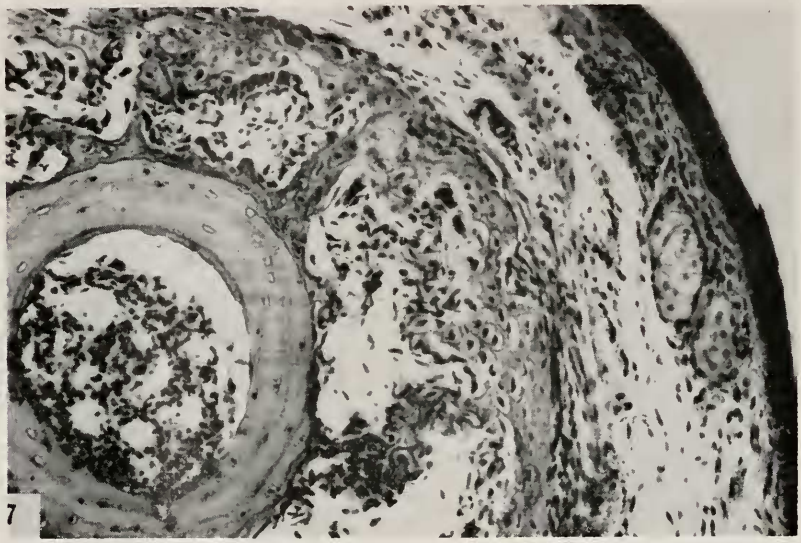


FIGURE 7. Photomicrograph of a cross-section from a digit (Case MCS 1, L-2-III) fixed thirteen days after amputation, showing the shape and extent of callus formation that follows amputation through the diaphysis. Observe the ring of new tissue formed around the old bone ring. External to the new layer of bone is a layer of large periosteal osteogenic cells. Between the peripheral region of the callus and the old bone new bone trabeculae, cartilage and blood elements are discernible (200 ×).

Amputation through and near the spongy epiphysis of the bone results in a different type of callus formation. Heavy proliferation of osteogenic elements is rare and, when found, these collect at the cut surface of the bone nearest to the diaphysis. Few cartilage cells are seen and remodeling appears to take place by osteoclasts and destruction of bone rather than by the formation of new bone trabeculae (Fig. 8, Case MCS 31, L-2-III).

Of particular interest in this and in other similar cases is the relative lack of reconstruction within the endosteal surface of the bone (Figs. 7 and 8), its main functions seeming to be confined to hemopoietic activities. Another interesting feature is the observation that the marrow cavity is invaded by connective tissue elements soon after amputation, and in these cases the processes of hematopoiesis revert to normal only after bone repair is completed.

*The formation of bursa-like cavities.* The aspects of post-amputational wound healing described so far concerned first the tissues immediately affected by ampu-

tation such as skin, and secondly the effects upon regions of the severed bone not directly injured by amputation. These are general reactions, encountered in any type of wound.

However, in amputational wounds of an appendicular organ such as a digit, features of healing are revealed which are peculiar and unique. Among these is the appearance of a pocket or tissue space which forms regularly over the end of the bone. At three days one observes a characteristic fluid area of necrotic debris and of free blood elements occupying the space between the cut bone and the covering epithelium (Fig. 4). With the onset of dermal healing, fibroblasts invade this area and five or six days after amputation the pool or pocket acquires a definite shape. The cavity or fluid space, similar to that seen in Figure 5 and which in many instances possesses a discrete synovial-like lining, closely resembles in histological features the bursae described by Black (1934).

Mention has been made in the literature of such an occurrence in amputated mammalian limbs: Nicholas (*op. cit.*) relates the formation of a bursa over the cut bone in a rat limb amputated in utero and fixed ninety days after birth, but no histological description was given. Nunnemacher (1939), studying the effects of partial amputation of epiphyseal cartilages in long bones of the same animal, describes and illustrates the appearance of a bursa within the connective tissues over the end of the cut bone. These observations are complemented by those of Urist, Mazet and McLean (1954) who describe the formation of a pseudo-arthroidal joint between the end of fractured bones that failed to appose. It does seem that a new bursa with a synovial-like lining is formed in amputational stumps in general, and that it is due to friction and irritation in much the same way as a pseudo-arthroidal joint is formed between the separated ends of a non-healing fracture.

Our observations differ from the above in the fact that the formation of a bursa-like cavity in amputated mouse digits was the rule rather than the exception: the fluid-filled area invariably appeared upon closure of the epidermis three days after amputation, acquired anatomical definition as a cavity in the midst of the invading fibroblasts, and then disappeared after the first post-amputational week. This last observation is in contrast to those made by the aforementioned authors in that bursal cavities here reported were transitory, while theirs were permanent.

*Terminal aspects of wound healing in a mouse digit and in a frog limb*

After the events described, the further post-amputational healing processes center around additional growth and thickening of the sub-dermal tissues. This latter thickening is effected by further fibrogenesis within the connective tissue, and the extreme degree of development of a pad of connective tissue in a digit fixed thirteen days after amputation is represented on Figure 8. The photomicrograph indicates that the digit was sectioned in a frontal, not a dorso-ventral plane, as the hair follicles are evenly distributed on both sides of the section. The figure shows that healing of the skin has been completed, the epidermal layers having reverted to their normal thickness; also, the capping of the end of the bone by the thick multi-layered pad of connective tissue suggests the end of growth of the cut phalanx. Observation of the dome-shaped connective tissue pad under higher magnification reveals its extensive vascular supply and also the "adult"

appearance of the blood vessels. As was explained above, bone callus formation is only slight in this case because amputation was effected near the proximal epiphyseal region; however, periosteal cells are in the process of chondrofication and some new bone trabeculae have appeared. For all intents and purposes healing has been completed and little proliferative activity remains. It is clear from

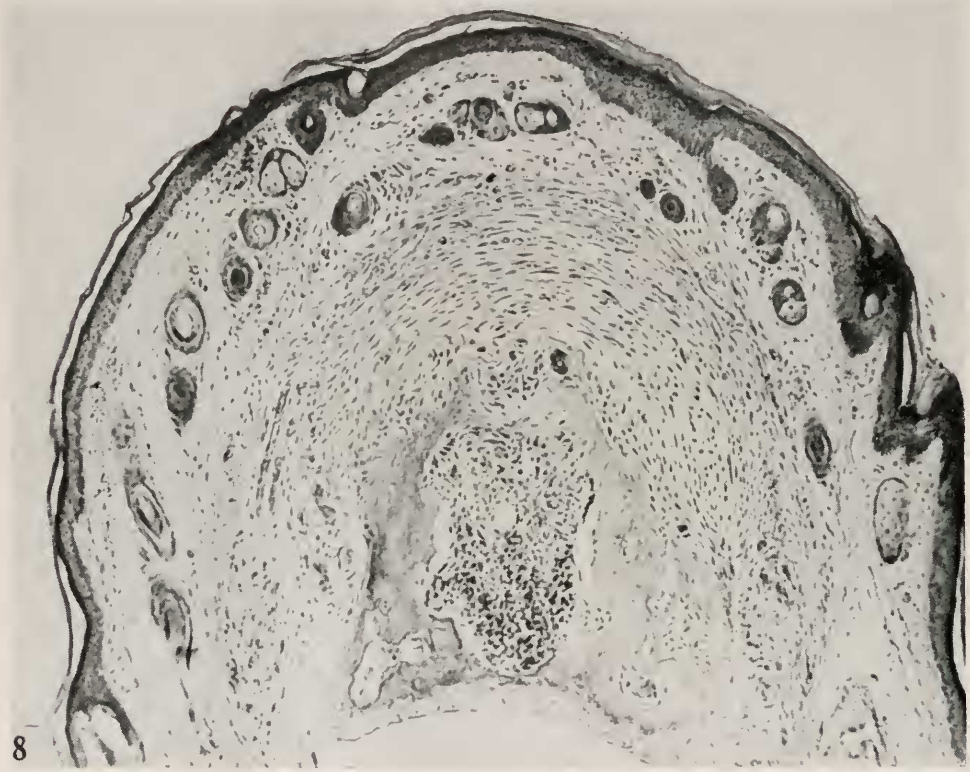


FIGURE 8. Photomicrograph of a longitudinal section from a digit (Case MCS 31, L-2-11) fixed 13 days after amputation, illustrating completion of epidermal and dermal healing. The epithelium has thinned out and has resumed a normal appearance together with the completely reconstructed dermis and sub-dermal layer containing numerous hair follicles. In addition a thick cap of concentric layers of fibroblasts has developed over the cut bone and it separates the injured tissues from the fully re-formed skin. Within the connective tissue cap numerous new blood vessels are distinguishable. Since amputation was near the epiphysis there is little callus formation (110×).

the examination of this and of similar slides from comparable post-amputational stages that no growth nor "regeneration" has occurred and that within a fortnight an amputated digital stump has reached true tissue equilibrium.

This "final" stage of wound healing observed in an amputated mammalian digit may be compared to advantage with a similarly "terminal" stage of amputational wound healing in another, also non-regenerating vertebrate, a frog. A section from a forelimb of a post-metamorphic frog (*Rana clamitans,* 4.5 cm. long

from snout to crotch), amputated through the lower arm and fixed 97 days after amputation will serve this purpose (Fig. 9). The longitudinal section from the lower forelimb shows: a completely regenerated skin as found on old amputational wounds; a cushion of sub-dermal formations including a fibroblastic pad tightly drawn over the terminal shaft of the ulna; finally, there is a well developed callus surrounding the severed shaft of the ulna.

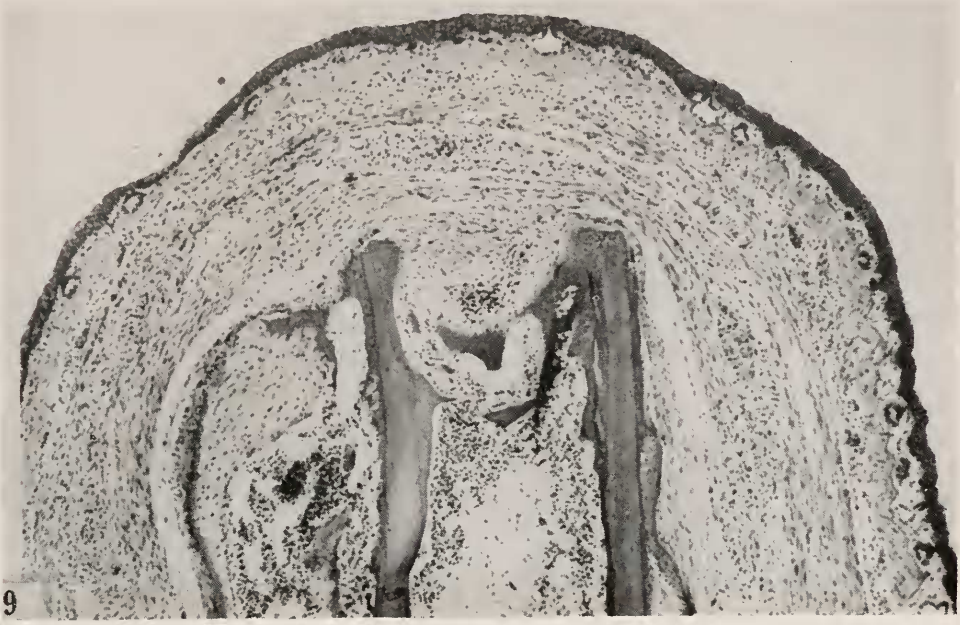


FIGURE 9. Photomicrograph of a longitudinal section from a left forelimb of a post-metamorphic *Rana clamitans* amputated through the lower arm and fixed 97 days after amputation. The skin at the amputation surface is fully reconstructed as shown by the numerous functional skin glands, a thick basal membrane; the quiescent status of the epidermis is evidenced by the normal number of cell layers. The amputated distal end of the ulna is capped by a callus, most prominent on the left side of the bone. The callus formations have become a fully integrated part of the ulna; the transformation of a large portion of the proximal portion of it into bone trabeculae is clearly visible on the left; moreover, parts of the still cartilaginous callus have acquired a periosteal bone collar and a functional periosteum as well. Distally from the callus and the bone shafts concentric layers of fibrous connective tissues intermingled with muscle fibers offer the characteristic aspect of a non-regenerating limb (60×).

Because of the appearance of bony differentiations at the proximal end of the callus where it fuses with the periosteum of the bony shaft of the ulna it is inferred that the growth phase of the appositional callus is terminated. This observation is also supported by the numerous sites of insertion of muscular bundles and of tendons visible on the newly formed periosteum. All the conditions of a terminated organogenesis and of a nearly completed histogenesis are fulfilled. That is to say, the frog limb after the severe amputational trauma inflicted a hundred days prior to fixation has reacquired the tissue equilibrium of a fully developed organ at rest.

## Recapitulation

The foregoing observations have shown that the healing processes following amputation in a mouse digit are unique, and that they differ from what has previously been described in non-amputational wound healing processes. This statement is based upon the following considerations.

After Marchand (*op. cit.*) it has been customary to classify skin wounds in two categories: firstly, those that show a small wound with little tissue loss and in which the epidermis heals over the wound without being preceded by granulation tissue formation are said to heal by primary intention; secondly, those that involve gross defects and extreme tissue loss involving particularly the dermis, heal more slowly, are characterized by accumulation of granulation tissue before epidermal healing may commence, and these are said to heal by secondary intention.

The wound produced by amputation of a digit certainly involves extensive tissue loss in an organ with a static morphological organization, and thus one would expect the features characteristic of a secondary type of wound healing. But all our observations concur to show precocious epidermal healing: as early as three days after amputation a completely joined epithelium was shown to exist; moreover, this epithelium was in many instances separated from the cut end of the bone by only a fluid area of tissue debris, and a completely re-formed epidermis was visible before any signs of dermal proliferation and repair had occurred. These features are certainly characteristic of primary wound healing, not of healing by secondary intention.

Lindquist (*op. cit.*), in an extensive study of skin wounds on the backs of rats, concluded that it was the contraction of the tissues around and beneath the wound which was more important for closure than was epidermal migration. Although the nature of the skin and of the soft tissues covering a mouse digit is different from that found on the back of a rat, our observations support the view of Lindquist that contraction plays an important role in the healing of these amputational wounds: while during the first day the amputation surface remains large (Fig. 1), by the third day of healing the wound surface is greatly reduced in size, and the actual extent of epithelial migration is small (Fig. 4). It also appears likely that the appearance of an amorphous fluid space between the healed epidermis and the cut bone is at least in part due to rapid epidermal closure. The subsequent organization of this space into a bursa-like cavity with its own lining is probably a secondary reaction to irritation within the amputation stump.

Another feature which distinguishes our observations from previously reported investigations concerns the bone callus. The illustrations shown offer evidence that in amputational wound healing the callus differs from that found in normally healing fractures. Following amputation through the diaphysis the formation of new bone trabeculae is irregular and diffuse thus producing a somewhat shapeless callus; however, amputation through the epiphysis results in mere destruction of the bone with little new callus formation. The irregular nature of the callus is no doubt due to the absence of a bone fragment in apposition to the injured stump, undoubtedly a condition which, in fractures, aids in the induction and organization of the proliferating elements into a more regular callus.

## Conclusion

The investigator of wound healing processes (surely an unsatisfactory term) cannot help but be impressed and awed with the organism's ability to respond in such a complex fashion to the stimulus of a simple amputation. The loss and destruction of tissues determined by infliction of a wound stimulates in some as yet unknown way (see the thoughtful discussion on the subject of "New Tissue Formation in an Adult Mammal" by Abercrombie, 1957) first of all the migration and proliferation of the various cells within the amputation stump; but, more particularly, it also reawakens processes characteristic of ontogenetically earlier stages that lead to organization of these new and old cells into morphogenetically distinct tissues and organs. Note, for example, the formation of the large bone callus, the heavy cap of connective tissues of the end of the bone, and the appearance of a walled-in bursa-like cavity, transitory as it may be. Every one of these reparative and morphogenic mechanisms is surely under some form of systemic control, be it "wound hormones," nerves or endocrines (Abercrombie, *op. cit.*).

While it is surely essential for the student of regenerative processes to understand the causative factors involved in this post-amputational proliferation and migration of cells, the comprehension of the reasons for their precocious and apparently "final" differentiation into equilibrated structures appears still more imperative. To one accustomed to observing the properties of regeneration in urodeles where organological equilibrium is achieved a long time after amputation, it is most important to understand why amputation of a mammalian appendage leads within such a limited time to that tissue and organ equilibrium which is the very essence of arrest of growth and development.

For these reasons we have thought it rewarding to compare post-amputational wound healing in a mammal, with the type of healing seen in the amputated limb of a post-metamorphic frog, an animal that before metamorphosis possessed the ability to regenerate amputated limbs. The comparison of the histological features of amputated limbs from two non-regenerating animals offers convincing evidence, we believe, of fundamental similarities in their patterns of wound healing. In fact, there is nothing in the histological appearance of either of these two appendages that would suggest that one of these non-regenerating limbs would be more susceptible to respond to treatments that might awaken regeneration than the other. Yet, workers of the past two decades have shown that limbs of post-metamorphic frogs can be induced to regenerate: by surgical trauma (Polejaiev, 1936), chemical trauma (Rose, 1944), by augmentation of nerve supply (Singer, 1954) and finally by altering the systemic hormonal balance (Schotté and Wilber, 1958).

In view of the success of these experiments with frogs, animals with wound healing patterns that are strikingly similar to those of mammals, it is legitimate to expect that modifications of at least some aspects of wound healing processes may also be observed in mice under the influence of some experimental devices. Some such responses in healing patterns of mouse digits have already been obtained and they will be reported in a forthcoming paper.

## Summary

1. A systematic study of the early stages of amputational wound healing in mouse digits is presented. Digits were regularly amputated through the middle

phalanx and a total of 191 cases were fixed for histological study at periods ranging from six hours to three weeks after amputation.

2. The first phases of post-amputational healing, characterized by provisional closure of the wound by a blood clot and by subsequent inflammation, were found to be similar to non-amputational wounds, but differences were observed in the patterns of epidermal, dermal and bone healing.

3. Epidermal healing began six hours after amputation and was completed in most cases by three days. Dermal and sub-dermal connective tissues showed the first signs of healing not earlier than three days after amputation and it was generally completed one week after amputation. The observation that epidermal closure of the wound preceded any signs of dermal repair indicates that these amputational wounds heal by "primary intention." It was also observed that tissue contraction contributed to this type of healing.

4. Depending upon the level through which the phalanx was amputated, two types of callus formations were observed: (a) amputation through the diaphysis resulted in the formation of a large callus that was more diffuse and amorphous than those previously described for healing fractures; (b) amputation through the epiphysis resulted in very little callus formation, often concomitant with destruction of bone.

5. An unusual aspect of amputational wound healing in mouse digits was the appearance of bursa-like formations between the cut bone and the healed epithelium; at three days a fluid space formed which subsequently developed into a structurally distinct cavity with the morphological characteristics of a bursa. However, these structures were only transitory and they disappeared during the second post-amputational week.

6. Two weeks after amputation the mouse digit was found to be almost completely healed. Additional growth in the form of a heavy cap of connective tissues arranged in parallel layers enclosing the distal and lateral parts of the cut bone was an invariable feature of this healing. The rapid return to equilibrium of the tissues within the amputation site was compared to a similar type of healing observed in amputated limbs of post-metamorphic frogs.

## LITERATURE CITED


Abercrombie, M., 1957. Localized formation of new tissue in an adult mammal. *Sym. Soc. Exp. Biol.*, **11**: 235–254.

Arey, L. B., 1936. Wound healing. *Physiol. Rev.*, **16**: 327–342.

Barron, D. H., 1945. The role of sensory fibers in the differentiation of the spinal cord in sheep. *J. Exp. Zool.*, **100**: 431–444.

Black, B. M., 1934. The prenatal incidence, structure and development of some human synovial bursae. *Anat. Rec.*, **60**: 333–355.

Blaisdell, F. E., and J. F. Cowan, 1926. Healing of simple fractures. *Arch. Surg.*, **12**: 619–654.

Cameron, G. R., 1952. Pathology of the Cell. Charles C Thomas, Springfield, Ill.

Clark, E. R., and E. L. Clark, 1953. Growth and behavior of epidermis as observed microscopically in observation chambers inserted in ears of rabbits. *Amer. J. Anat.*, **93**: 171–219.

Ham, A. W., and W. R. Harris, 1956. Repair and transplantation of bone. *In:* The Biochemistry and Physiology of Bone. G. Bourne, ed., 475–505. Academic Press, N. Y.

Harkins, H. N., 1957. Wound Healing. *In:* Surgery. J. G. Allen, H. N. Harkins, C. A. Moyer, J. E. Rhoads. pp. 7–28. J. B. Lippincott, Philadelphia.

LEVANDER, G., 1950. On the epithelium regeneration in the healing of wounds. *Acta Chir. Scand.*, **100**: 637–649.

LINDQUIST, G., 1946. The healing of skin defects. *Acta Chir. Scand.*, **94**, Suppl. 107: 1–163.

LOEB, L., 1898. Über Regeneration des Epithels. *Arch. f. Entw.*, **6**: 297–364.

MARCHAND, F. J., 1901. Der Process Wundheilung. Deutsche Chirurgie, Lief. 16.

MAXIMOW, A. A., AND W. BLOOM, 1957. A Textbook of Histology. W. B. Saunders Co., Philadelphia.

MCINTOSH, D., 1954. A study of the processes of post amputational wound healing in the limbs of young rats under various experimental conditions. Thesis, Amherst College.

MCLEAN, F. C., AND M. R. URIST, 1955. Bone. Univ. of Chicago Press.

NICHOLAS, J. S., 1926. Extirpation experiments upon the embryonic forelimb of the rat. *Proc. Soc. Exp. Biol. and Med.*, **23**: 436–439.

NUNNEMACHER, R. F., 1939. Experimental studies on the cartilage plates in the long bones of the rat. *Amer. J. Anat.*, **65**: 253–289.

POLEJAIEV, L. W., 1936. Sur la restauration de la capacité régénérative chez les Anoures. *Arch. Anat. micr.*, **32**: 437–463.

ROBBINS, S. L., 1957. Textbook of Pathology. W. B. Saunders Co., Philadelphia.

ROSE, S. M., 1944. Methods of initiating limb regeneration in adult Anura. *J. Exp. Zool.*, **95**: 149–170.

ROSE, S. M., 1948. Epidermal differentiation during blastema formation in regenerating limbs of *Triturus viridescens*. *J. Exp. Zool.*, **108**: 337–361.

SCHOTTÉ, O. E., AND J. F. WILBER, 1958. Effects of adrenal transplants upon forelimb regeneration in normal and in hypophysectomized adult frogs. *J. Embryol. and Exp. Morph.*, **6**: 247–261.

SINGER, M., 1954. Induction of regeneration of the forelimb of the postmetamorphic frog by augmentation of the nerve supply. *J. Exp. Zool.*, **126**: 419–472.

SLOCUMB, D. B., AND D. R. PRATT, 1944. The principles of amputations of the fingers and hand. *J. Bone and Joint Surg.*, **26**: 535–546.

URIST, M. L., R. MAZET AND F. C. MCLEAN, 1954. The pathogenesis and treatment of delayed union and non-union. *J. Bone and Joint Surg.*, **36A**: 931–967.

WEISS, P., 1959. Biological aspects of wound healing. *In:* Wound Healing and Tissue Repair. W. B. Patterson, ed., Univ. of Chicago Press.